CHAPTER 7

General discussion

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Cardiovascular disease is one of the most serious and expensive morbidities in the western population. Patients with cardiovascular disease are at higher risk for a number of comorbidities, including diabetes and renal failure. Another large group of comorbidities found in patients with cardiovascular disease is mental dysfunction. This group of morbidities encompasses several pathologies, including depression, anxiety and cognitive impairment.

Even though these comorbidities are often overlooked, seen as harmless side-effects, or regarded as a "natural response to a life-threatening condition", it is known that patients that have both cardiovascular disease and mental dysfunction have an unfavourable outcome compared to patients with cardiovascular disease that do not suffer from mental dysfunction [1, 2].

While the knowledge concerning the seriousness of mental complications in heart disease is growing, prevention and treatment options are still very limited. We know for instance that, although cardiovascular disease with comorbid depression worsens prognosis, treating myocardial infarction (MI) patients for depression, does not improve their cardiovascular prognosis [3].

What is needed to successfully treat cardiovascular patients for their mental complications is, apart from more awareness, more knowledge about the pathophysiology of these complications. Studies in patients have found several risk factors for mental dysfunction in cardiovascular patients, including gender, age, and lifestyle factors [4, 5]. Additionally, inflammatory factors have been linked to mental dysfunction in patients with cardiovascular disease. Heart failure and depression share increased levels of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6 and CRP [6-9]. Recently we also found that neutrophil gelatinase associated lipocalin (NGAL) was associated with depression in heart failure patients [10]. Results from our later study revealed that NGAL was associated with other markers of inflammation, including TNF-α, and remained a significant marker for somatic symptoms of depression in heart failure patients after correction for these inflammatory factors [11]. These studies on NGAL and other inflammatory markers formed a motivation for the experimental studies described in this thesis.

The goal of the research culminating in this thesis was to investigate the role of (neuro) inflammation in mental dysfunction in heart failure. Experimental models of MI in mice and rats were used to study the connection between cardiovascular damage, neuroinflammation

and behaviour. To study (neuro)inflammation, we focussed on microglia, as the immune cells of the brain; the TNF-α pathway, as a well-known pathway involved in depression and NGAL, as a novel marker that may link cardiovascular disease and depression.

Microglia

Microglia are resident immune cells located in the central nervous system and are mostly known for this function. They can be activated by infection, trauma, normal ageing and other stimuli. Microglia also play an important role in the development of the brain, shaping neuronal networks by pruning of synapses [12, 13]. Activated microglia are capable of secreting inflammatory cytokines and other active molecules and have been reported to have the ability of phagocytosis. Microglia activation serves a protective role, responding to pathogens and assisting in the repair of injury. There are, however, also indications that excessive microglia activation could be involved in neurodegeneration and psychiatric disorders, including depression [14, 15]. PET studies in patients showed increased microglia activation post-MI [16].

Resting microglia consist of a small cell body and long branched processes for scanning the environment for signs of danger. Classically, microglia activation is measured as morphological changes; the processes thicken and retract and the cell body increases in size. However, other morphological changes in microglia have also been documented [17]. Dystrophic microglia, for example, are characterized by irregular cell body shape, interrupted processes and debris [18], and are thought to be senescent or degenerated. We measured microglia activation in a mouse model and rat model of MI. In mice we found microglia activation in the hippocampus [19], whereas in rats we found no difference in microglia number or activation between sham and MI (Gouweleeuw et al., unpublished results). These results are inconsistent with previous findings. Others have found microglia activation in the paraventricular nucleus of the hypothalamus (PVN) in rats that underwent MI surgery [20, 21]. Rinaldi et al. also found increases in the number of activated microglia in cortex and hippocampus of MI rats versus control, and an increase in the number of dystrophic microglia in the cortex, thalamus and PVN following MI [22]. Important to note is that this last study compared MI rats to home cage controls, while our studies always compared MI to sham operated animals. A previous study from our group found microglia activation in MI rats compared to controls, but not compared to sham operated animals, indicating sham surgery already causes microglia activation [23]. Another explanation for the apparent lack to show upregulation of microglia activation in our MI model might be a timing issue. In a

model of abdominal surgery, microglia activation was shown one week after surgery, but returned to normal after 2 weeks [24].

Microglia activation, in some degree, was associated with behavioural parameters in male rats (Gouweleeuw et al., unpublished results). Microglia activation in amygdala was associated with lower degree of exploration and lower motivation for exploration, while microglia activation in the PVN was also associated with lower motivation for exploration. Interestingly, these associations were not found in female rats. Here we did observe that microglia activation in the hippocampus correlated to anxious behaviour in the elevated plus maze. Even though these correlations could be regarded as indication that microglia activation is linked to behavioural outcome, this effect is not specific for MI, since we found the same associations in sham and MI animals. Probably the damage inflicted by the sham surgery itself already causes microglia activation, which is not exacerbated by the MI procedure. More likely, microglia activation following physical damage in general is linked to behaviour. This is also apparent from a study in a model of abdominal surgery, where we also found microglia activation linked to behavioural parameters [25].

TNF-α

TNF-α is one of the cytokines that shows a large increase following MI, in both animal models and in patients. In animal models of MI, TNF-α expression in the brain is also upregulated compared to sham operated animals. Moreover, in patients, increased TNF-α levels are associated with depression [8]. This is also true for depression in heart failure patients [11]. These previous findings make TNF-α an interesting candidate in the study of neuroinflammation and behaviour following MI.

We measured brain TNF-α through western blotting and immunohistochemical staining in male sham and MI mice and found an increase in TNF-α combined with a trend towards higher pro-inflammatory TNF-R1 and lower cytoprotective TNF-R2 expression in MI versus sham mice with western blotting. This could indicate a shift to a more pro-inflammatory state. Staining TNF-α in brain sections did not show significant differences between MI and sham in the PVN, piriform cortex and prefrontal cortex. At 14 days post-MI, no difference was found in circulatory cytokines, including TNF-α, between sham and MI mice [19]. In rats, TNF-α in plasma and CSF was measured in male and female sham and MI rats. In male rats there was a 3-fold increase in TNF-α in plasma, while the TNF-α in CSF was below a 2-fold change. In female rats, no differences were found (Gouweleeuw et al., unpublished results).

The results on TNF-α partially overlap with previous findings by others. Increased expression of TNF-α in the brain and circulation after experimental MI was found also by others [26]. However, the lack of any cytokine increase 2 weeks after the MI in mice is surprising. Other people have observed an increase of pro-inflammatory cytokines post-MI in mice [27]. An issue here could be that timing of the measurements, as another study also found no differences 6 or 7 days post-MI when compared with sham animals [28].

To summarize, TNF-α likely plays a role in post-MI neuroinflammation, even though circulatory TNF-α was not consistently elevated in our animal models.

NGAL

A relatively novel marker in this field is Neutrophil gelatinase associated lipocalin (NGAL). NGAL is an inflammatory marker that serves an antimicrobial function as part of the innate immune system, at least in part through its function in iron regulation [29, 30]. However, its expression is associated with several disease states, and is seen as a biomarker. It is well known for its prognostic value in patients with heart failure [31, 32]. A previous clinical study from our group showed that NGAL levels were increased in elderly patients with depression [33]. Moreover, our earlier study in heart failure patients showed that NGAL was associated with depressive symptoms in heart failure patients, and that this was independent of left ventricular dysfunction or other (inflammatory) factors [10, 11]. This may indicate that NGAL is an associative marker between cardiovascular disease and depression.

Interestingly, NGAL was highly upregulated in neurons stimulated with TNF-α, as shown by oligonucleotide array [34]. This study also showed that this upregulation was mediated by pro-inflammatory TNF-R1 and that NGAL downregulates the cytoprotective TNF-R2, thereby limiting the neuroprotective effects of TNF-α.

As previous research from our group would support the possible role of NGAL as a link between heart disease and depression [10, 11], NGAL expression in sham and MI rats and their association with behavioural parameters was studied [35]. We found gender and MI effects on plasma and CSF NGAL, with NGAL being higher in MI vs sham and higher in male vs female rats. No significant differences of NGAL expression were found between the groups in the hippocampus and hypothalamus. We also found that NGAL expression in the circulation, CSF and brain correlated with each other. An NGAL staining in the PVN further showed that in male rats, MI led to an increase in the number of NGAL positive cells in the magnocellular part of the PVN, while no differences were found in female rats [35].

Furthermore, in male rats, we observed correlations between NGAL expression and disease parameters. Higher NGAL was associated with higher heart weight, lung weight and infarct size. This effect was not found in female rats. In addition, an association between NGAL and behaviour was observed in male rats. Higher plasma NGAL was associated with lower distance in the open field, lower motivation to explore and decreased special memory. Hippocampus and PVN NGAL were only associated with distance in the open field. In the female rats, we only found hippocampus NGAL correlated with open field distance, but we did not find any significant correlations between plasma and PVN NGAL and behavioural parameters.

We were not the first to show an increase in NGAL in experimental MI. It was already reported that in male rats, NGAL in the heart was increased up to 64 days post-MI [36]. NGAL increase in the brain or associations with behaviour in an MI model were, to the best of our knowledge, not previously reported.

In order to indicate a more general phenomenon regarding the function of NGAL, we investigated NGAL and behaviour in a different disease model, post-operative cognitive decline (POCD). Here we found that plasma and hippocampal NGAL were increased 6 weeks after surgery and that higher hippocampal NGAL was associated with decreased exploratory behaviour and lower spatial learning ability [25]. These results are interesting, as they show that NGAL can be a mediator between physical damage and cognitive function. This might indicate NGAL would be a good general marker for neuroinfammation following physical damage.

Clinical implications

In this thesis we showed evidence for neuroinflammation after MI and its association with behavioural parameters. While others support inflammatory parameters linked to depression and cognitive decline in patients with cardiovascular disease [8, 37, 38], and earlier studies from our group also focussed on patients [10, 11], this thesis describes results found in experimental models of MI.

Gender differences in MI induced inflammation and behavioural changes are observed. In patients with cardiovascular disease, women are more prone to develop mental complications [4, 39]. In our rats, we found that males had both more inflammatory response and more behavioural changes compared to females. Recently, another MI study in rats also observed behavioural differences in male MI rats, but not in females [40]. In female MI rats, mild

depressive-like symptoms were found when females were ovariectomized. Estrogen supplementation prevented the depressive-like symptoms. Estrogen replacement also diminished cytokine upregulation in the prefrontal cortex after MI. This suggests that in rats, estrogen is responsible for the protection against MI induced inflammation and mental dysfunction. However, in a pilot study in old (>18 months) female rats with no clear cycle anymore, no depressive-like behaviour nor cognitive decline could be observed. Nevertheless, post-MI mortality was high (80%) rendering survivors as potentially not representative (Liu et al., unpublished results).

In patients with non-obstructive coronary artery disease we do see signs of gender differences in the association between inflammatory markers and depression and anxiety (Mommersteeg et al., in press). Small effect sizes were found for correlations between inflammatory markers and depression/anxiety symptoms, which did not hold after correction for multiple testing. The association between BDI test cognitive signs of depression and hsCRP showed an interaction effect, where only in men depression was associated with hsCRP levels, while not in women. These results combined with previous publications from us and others show that there is evidence that the correlation between inflammation and mental dysfunction could be gender specific. The relationship is likely complicated though, as patient and animal studies don't always point in the same direction, limiting translational opportunities.

Future perspectives

While there is an increasing acknowledgement for the impact of mental dysfunction in MI patients, its treatment is still difficult. As previously mentioned, treating cardiovascular patients for their depression with the regular anti-depressant drugs does not improve prognosis. In rats it was found that pharmacological treatment to improve cardiovascular function was not simply associated with an increase in quality of life, measured as behavioural outcome [41]. Similarly, in patients this effect was seen, as mental function does not improve upon proper pharmacological therapy for cardiovascular symptoms [42]. In women with post-MI depression, it has been reported that treatment with anti-depressant drugs even worsens prognosis, indicating depression might serve a protective role [43]. Following results from previous studies with anti-depressant drugs, it is clear that different treatment options should be developed.

Although we now have evidence that inflammation could be linked to symptoms of depression as well as to heart failure in patients, this may be a common underlying mechanism rather than causing either of the conditions. Still, inflammation could be a possible target for

treatment. There are, however, difficulties in treating patients with anti-inflammatory drugs. Targeting TNF-α in animal models of ischemia reperfusion and chronic MI seemed promising, as it diminished cardiac injury [44, 45], as well as it improved behavioural outcome [46]. There has been some evidence that use of TNF-α inhibitors is associated with lower incidence of depression in patients with rheumatoid arthritis [47]. However, multiple studies of TNF-α inhibition in patients with cardiovascular disease failed to show improvement, making TNF-α inhibition an unlikely treatment option for this group of patients [48-50]. Finally, some degree of inflammation is necessary for wound healing and scar formation in the myocardium after MI, challenging the pro/anti-inflammatory balance.

NGAL looks promising as a prognostic marker. It has been linked to depression in the elderly, depression among heart failure patients and all-cause mortality in heart failure patients [10, 32, 33, 51]. When going to a more individual approach to treating cardiac patients, patients with higher NGAL levels might benefit from a treatment aimed at preventing and treating cognitive dysfunction including depression. This will probably have to be done in combination with other markers, since NGAL has been linked to a variety of diseases and complications, including renal disease, cancer, intestinal inflammation and metabolic syndrome [31, 52-54]. It might be too soon to speculate whether NGAL could be used as a potential target for therapy, since it has several important functions in the body and we have not totally unravelled its actions.

In conclusion, we showed different behavioural and (neuro)inflammatory changes in our animal models of MI, suggesting cognitive dysfunction associated with cardiovascular disease goes beyond psychological factors. However, this research only indicates that there is an association between (neuro)inflammatory factors, cardiovascular function and behaviour. Whether there is a causal relationship, still needs to be determined. Future research should focus on finding new treatment strategies to combat cognitive dysfunction in patients with cardiovascular disease, as present therapeutic options are still very limited. As treatment with conventional anti-depressant drugs and drugs to improve cardiac function doesn't seem to give the best prognosis, it could be time to look into different targets. Inflammation is one of the possible candidates, as we know inflammatory markers are linked to behavioural outcome following cardiovascular disease in both animal and patient studies. Yet, which specific hallmarks of inflammation should be targeted, has to be investigated further. Regarding the complex character of the heart-brain interaction, a more general anti-inflammatory approach rather than specific blockade of one inflammatory factor or receptor seems preferable. Physical exercise for instance has well-known anti-inflammatory effects, positive effects in

cardiovascular disease as well as in depression, without negative side effects. Complicating factor though is that cardiac patients are not eager to exercise because they are concerned about their heart, and depressed patients cannot find the motivation to exercise, let alone depressed heart failure patients. Alternatives to physical exercise, like whole body vibration, could be investigated as a therapeutic option, though these alternatives will first have to be tested for their effects on inflammation and cognition in this specific group of patients.

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